Damage of cellular material under simultaneous application of pressure and pulsed electric field

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Influence of pulsed electric field (PEF) simultaneous to pressure treatment on moisture expression from fine-cut cellular raw material has been investigated. Dependencies of specific conductivity σ, liquid yield Y, instantaneous flow rate v and qualitative juice characteristics at different modes of PEF treatment are discussed. Three main consolidation phases were observed in a case of mechanical expression. A unified approach is proposed for liquid yield data analysis allowing to reduce the data scattering caused by differences in the quality of samples. Simultaneous application of pressure and PEF treatment allowed to reveal a passive form of electrical damage. Pressure provokes the damage of defected cells, enhances diffusion migration of moisture in porous cellular material and depresses the cell resealing processes. PEF application at a moment when a sample specific electrical conductivity reaches minimum and pressure achieves its constant value seemed to be the most optimal.

Notation

d	mean cell's dimension, μm
D	diffusion coefficient, $m^2\ s^{-1}$
E	electric field strength, kV cm^{-1}
k	area normalizing coefficient
M^1	first Moment, s
N	number of pulses
P	pressure, bar
S	area under the expression curve $Y(t)$, % s
t_i	pulse duration, μs
t_{max}	maximal time of pressing, s
t_p	time of PEF treatment application, s
$t_{v_{max}}$	time, where maximum of instantaneous flow rate is observed, s
Δt	pulse repetition time, ms
T	temperature, K
v	$= dY/dt$ instantaneous flow rate, % s^{-1}
U	external voltage, V
W	moisture content, %
Y^I	first normalized form of liquid yield
Y^{II}	second normalized form of liquid yield
Y^*	intensification degree of PEF treatment

Greek letters

σ	conductivity, S m^{-1}
τ	characteristic time of expression, s
τ^*	coefficient of PEF enhanced durability
τ_D	$\sim d^2/6D$ time constant of diffusion process, s

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Subscripts

E	with PEF treatment
$E = 0$	without PEF treatment
r	reduced to the maximal value
∞	in the limit of infinite time

Abbreviations

PEF	pulsed electric field
SEM	scanning electron microscopy

I. INTRODUCTION

Mechanical expression (hydraulic pressing) is widely used in the processes of solid-liquid separation for extraction of fruit juices and vegetable oils, dewatering of fibrous materials, etc. (Schwartzberg, 1983). Efficiency of this process can be increased by raw material plasmolysis, cellular damage or permeabilization prior to its expression. Different methods are traditionally used to increase the degree of raw material plasmolysis: heating, osmotic drying or freezing dehydration, alkaline breakage, enzymatic treatment, etc. (Rao & Lund, 1986; Aguilera & Stanley, 1999; Tsuruta, Ishimoto & Masuoka, 1998; Ponant, Foissac & Esnault, 1988; Jones, 1988; Barbosa-Cánovas & Vega-Mercado, 1996). Earlier on, the method of electric field treatment (both d.c. and a.c.) was also proposed for cellular material plasmolysis (known as electro-plasmolysis). The methods of electro-plasmolysis were shown to be good for juice yield intensification and for improving the product quality in juice production (Scheglov, Koval, Fuser, Zargarian, Srimbov, Belik et al., 1988; McLellan, Kime & Lind, 1991, Bazhal & Vorobiev, 2000), processing of vegetable and plant raw materials (Papchenko, Bologa, Berzoi, 1988; Grishko, Kozin, Chebanu, 1991), food stuffs processing (Miyahara, 1985), winemaking (Kalmykova, 1993), and sugar production (Gulyi, Lebovka, Mank, Kupchik, Bazhal, Matvienko et al., 1994; Jemai, 1997). But all these electric field applications are usually restricted by the high and uncontrolled increase in cellular tissue temperature and product quality deterioration because of electrode material electrolytic reactions, etc.

Recently, a variety of new high and moderate pulsed electric field (PEF) applications were successfully demonstrated for liquid and solid foods (Barbosa-Cánovas, Pothakamuri, Palou & Swanson, 1998; Wouters & Smelt, 1997; Knorr, Geulen, Grahl & Sitzmann, 1994; Knorr & Angersbach, 1998, Barsotti & Cheftel, 1998). The PEF application provides a possibility of fine regulation of electric power input and may result in effective permeabilization of cellular membranes (Zimmermann, 1975; Chang et al., 1992; Weaver & Chizmadzhev, 1996) without significant temperature elevation (Barbosa-Cánovas et al., 1998).

One of emerging and promising method is the combined PEF and pressure application, which demonstrates significant yield intensification for juice extracted from apples and beets and clarification of the extracted juice (Vorobiev, Bazhal & Lebovka, 2000; Gulyi et al., 1994). But the major problem arising from simultaneous application of mechanical expression and PEF treatment is the choice of optimal modes of treatment. The mechanism of solid/liquid expression from cellular materials is rather complex and may include many different phases of consolidation process (Lanoiselle, Vorobiev, Bouvier & Piar, 1996). The electric breakdown of a cellular system can influence consolidation phases and change drastically the expression curves. Unfortunately, up to now there are no accepted mechanism of electric breakdown in the cellular systems and reliable criteria for choosing optimal parameters of electric field treatment (Lebovka et al., 1996, Lebovka, Bazhal & Vorobiev, 2000). Another problem is the poor reproducibility of the experimental data, which is typical for objects of biological origin.

The properties of cellular materials influence significantly the the electrotreatment efficiency. The electrometry can be used for characterization of changes in tissue properties under the influence of external factors (electric field, pressure). This is a simple method, as far as the electrical conductivity, σ, reflects a degree of a water saturated tissue permeability (Sahimi, 1995). But the general dependence between the structure of a cellular material and σ may be rather complex, because the conductivity of a biological tissue may be influenced by a number of processes, such as resealing of membranes in cells (Heinz, Angersbach & Knorr, 1999), diffusional redistribution of moisture inside the samples, etc.

The objective of this study is the optimization of moisture expression from biological raw materials under simultaneous pressing and PEF treatment.

The liquid expression from fine-cut cellular tissue after PEF application for different modes and durations of precompression has been investigated. Apple was used as the example of cellular material.

The useful method of data treatment, which allows to reduce data scattering caused by differences in quality of the samples is described. Discussion of the consolidation kinetics before and after PEF treatment is also given.

II. PRELIMINARY REMARKS

The reasons for the simultaneous application of mechanical expression and PEF treatment are as follows. The excessive quantity of extraparticle liquid and absence of contacts between solid particles increases electrical energy losses. So the effectiveness of the PEF treatment is restricted by the uniform and tight packing of raw material between electrodes and previous removing of extraparticle air and excessive liquid (from cells destroyed by cutting). The method of raw material compact formation is its pre-consolidation. Moreover, effectiveness of the PEF treatment with respect to the water-saturated cellular materials is restricted by the low values of moisture content, W. The PEF treatment is ineffective for a water-saturated system (which is the case for the fine-cut apple raw material) because of electric breakage and uncontrolled increase of current flow through the system. The initial steps of consolidation remove an excess liquid from the extracellular volume. Therefore, we can expect increase of the PEF treatment efficiency after pre-consolidation of the raw material.

III. MATERIALS AND METHODS

A. Preparation of apple slices

Freshly harvested apples of Golden Delicious variety were selected for investigation and stored at 4°C until required. The moisture content of apples W was within 80-85%. The fine-cut apple pieces (3-5 mm diameter) were prepared from an apple pap using rasp.

B. Experimental setup and instrumentations

Figure 1 is a schematic representation of the experimental set-up. All experiments were carried out using laboratory filter-press cell equipped with an electrical treatment system. The polypropylene frame had a cylindrical cavity compartment (20 mm thick, 56 mm in diameter). The cavity compartment of frame was initially filled up with apple slices and then was tightly closed from both sides by the steel plates. One of the plates, covered by a filter cloth, was used as a stationary electrode. The other plate was attached with an elastic rubber diaphragm. A mobile wire gauze electrode was installed between the diaphragm and the layer of apple slices. Pressure was applied to the layer of apple slices through the elastic diaphragm using the hydraulic pressure controller GDS 'Standard' (GDS Instruments Ltd, UK) with water as a pressure fluid. The pressure controller provided a constant pressure from 1 to 30 bars. The yield of liquid was controlled by balance PT610 (Sartorius AG, Germany).

A high voltage pulse generator, 1500V-15A (Service Electronique UTC, France) provided the monopolar pulses of rectangular shape and allowed pulse duration t_i varied within the interval of $10 - 1000$ μs (to precision ± 2 μs), pulse repetition time Δt within the interval of $1 - 100$ ms (to precision of ± 0.1 ms) and number of pulses n within the interval of $1 - 100000$. The conductivities were measured by contacting electrode method with an LCR Meter HP 4284A (Hewlett Packard, 38 mm guarded/guard Electrode-A HP 16451B) for thin apple slice samples at the frequency of 100 Hz and with Conductimetre HI8820N (Hanna Instruments, Portugal) for the apple juice samples at a frequency of 1000 Hz (these frequencies were selected as optimal in order to remove the influence of polarising effect on electrodes and inside the samples). Pulse protocols and all the output data (current, voltage, impedance, pressure, juice yield and temperature) were controlled using a data logger and special software HPVEE v.4.01 (Hewlett-Packard) adapted by Service Electronique UTC, France).

High resolution scanning electron microscopy (SEM) images were obtained using the instrument XL30 ESEM-FEG (Philips, V=15 kV, P=3.5 Torr). The "WET" chamber mode allowing observation of hydrated apple specimens in their natural state was applied.

The optical absorbance of an expressed liquid was measured with Photocolorimeter CO75 (WPA Ltd, UK) at the wavelength 520 nm. The characteristic absorption spectra were determined with respect to distilled water. Transmittance of an expressed liquid was calculated as a ratio of filtered and nonfiltered liquid absorptions. The liquid was filtrated using a Whatman 2V filter paper.

C. Methods

All experiments were done using electric field voltages U from 200 to 1500 V, pulse duration $t_i = 100$ s, pulse repetition time $\Delta t = 10$ ms, number of pulses $N = 50$, constant pressure $P = 3$ bars and total time of mechanical

expression t up to 10^4 s. Pressure value of 3 bars was accepted as the most efficient for exhibition of the effect of simultaneous pressing and PEF treatment (Vorobiev et al., 2000). The experiments were repeated, at least, five times.

IV. RESULTS AND DISCUSSION

A. Phases of consolidation

Figure 2 presents typical experimental curves of liquid yield Y_r, instantaneous flow rate v_r, pressure P_r and specific electrical conductivity σ_r vs. time t. For convenience of presentation, here all properties are reduced to their maximal values, e.g., $Y_r = Y/Y_{max}$, etc., and flow rate is determined as $v = dY/dt$.

Initially we observe a rather rapid increase of liquid yield Y, and decrease of electrical conductivity, σ. This behaviour corresponds to the layer pre-compaction (at a constant velocity of elastic diaphragm displacement) and expulsion of extraparticle air-liquid mixture. The maximum of instantaneous flow rate v is observed approximately at $t = t_{v_{max}} = 50 - 60$ s. In the absence of PEF treatment ($U = 0$, or $E = 0$) the curve of $\sigma(t)$ temporarily stabilises in time interval $300 < t < 1000$ s and by this moment the pressure P reaches its maximal value of 3 bars. This behaviour correspond to the end of pre-compaction period. Then, at $t > 1000$, the $\sigma(t)$ curve ($E = 0$) slightly rises, which can be explained by mechanical rupture of residual cells, by the material deterioration and by the effects of the biological activity of microorganisms.

Typical SEM micrographs of apple tissue structure before and after pressing are presented in Figs. 3(a) and (b), respectively. The mean size of undamaged cells is of order $100 - 200$ μm. We see, that after pressing some of the cells are destroyed, but there exist also intact cells. So, for a given mode of treatment ($P = 3$ bars, $t = 600$ s) the cellular structure is not completely disrupted after pressing and there exists some isolated cells, which remain intact during pressing period. These cells can be damaged or partially permeabilized by another methods, for example, by PEF, thermal or another mode of treatment.

In analysing the results presented in Figs. 2, 3(a,b) we can discern the following phases of press-cake layer consolidation process:

- Phase I. Initial compaction of press-cake and expulsion of air-liquid mixture, or pre-consolidation period ($0 < t \lessapprox 2t_{v_{max}} \cong 100 - 120$). During this phase the velocity of elastic diaphragm displacement is constant and a maximum of liquid flow rate is observed.
- Phase II. Mechanical rupture of cells and expulsion of liquid from ruptured cells ($100 - 120 \lessapprox t \lessapprox 300 - 400$). The decrease of both liquid flow rate and velocity of elastic diaphragm displacement and acceleration of the pressure increase are observed.
- Phase III. Final consolidation of press-cake at constant pressure, packing of press-cake and retardation of liquid flow rate ($100 - 120 \lessapprox t$). The liquid flowing from intracellular, extracellular and extraparticle volumes is expressed from the press-cake. At the beginning of this phase a minimum value of the specific electrical conductivity is observed. Moisture occupies all channels and so the press-cake is said to be in an impregnated state.

These phases are shown schematically at the top of Fig. 2.

B. Methods of data analysis for combined pressing and PEF treatment

Significant changes in kinetics of moisture expression and press-cake consolidation can be observed after PEF treatment. Figure 4(a) presents some examples of experimental curves of liquid yield $Y(\%)$ versus time t. It can be seen that $Y(t)$ curves rise significantly after PEF application (applied in this case at $t = 600$ s) as the result of damage or partial permeabilization of intact cells and subsequent expression of liquid.

But here, the main problem is in poor reproducibility of the experimental $Y(t)$ data. The measured curves of $Y(t)$ can deviate substantially because of differences in initial humidity of samples. This difficulty can be overcome by consideration of the normalized or reduced liquid yield. This normalization procedure was executed in two steps. We began with the first normalized form of liquid yield defined as (see Fig. 4(b)):

$$Y_E^I = Y_E(t)/Y_E(t_{max}), \tag{1}$$

where t_{max} is the maximal time of pressing (here we use the value $t_{max} = 5400$ s), Y_E values correspond to the values of Y at different electric field strengths E.

We assume that all liquid yield curves should be equal in the time range of $t < t_p$ for identical conditions of pressing. For experiments with PEF application ($E \neq 0$), we take the curve $Y^I_{E=0}(t)$ for $E = 0$ as a reference. Then we calculate the area under this curve for the time period $t < t_p$ and renormalize all the curves $Y^I_E(t)$ so as to obtain the same values of

$$S_E = \int_0^{t_p} Y^I_E(t)dt, \tag{2}$$

for all curves.

Normalization coefficient is given by

$$k_E = S_{E=0}/S_E. \tag{3}$$

The second normalized form of liquid yield curve is defined as follows (see Fig. 4(c)):

$$Y^{II}_E(t) = k_E \cdot Y^I_E(t). \tag{4}$$

The degree of intensification caused by PEF treatment, Y^* can be determined as the following ratio of the second normalized forms for pressing with and without PEF treatment:

$$Y^* = Y^{II}_E(t_{max})/Y^{II}_{E=0}(t_{max}). \tag{5}$$

Here the values of $Y^{II}_{E,E=0}(t_{max})$ were determined in the point of the maximal time of pressing $t = t_{max}$.

Another interesting property of the expressing is the mean characteristic time, which characterizes durability of the process. The theory developed for mechanical expression of cellular materials (Lanoiselle et al., 1996) describes expression process with the set of characteristic times for the different expression phases. These are very valuable characteristics of the expression processes but, in practice, it is very difficult to find them proceeding from the expression curves. The main problem here is that we can never determine the exact value of limiting expression quantity Y_∞ at $t \to \infty$. For vegetable stuff we are faced with the problem of high enzymatic destruction at continuous expression. So we always stop the expression process at rather long, but finite time (in our case we choose $t_{max} = 5400$ s) and the obtained values of $Y(t_{max})$ are of course less then the actual values of Y_∞. More general approach implies evaluation of the first Moment of the function $F(t) = Y(t_{max}) - Y(t)$:

$$M^1 = \frac{\int_0^{t_{\max}} F(t)tdt}{\int_0^{t_{\max}} F(t)dt} \tag{6}$$

It is easy to show, that for the simple exponential function $F(t) = \exp(-t/\tau)$ and $t_{\max} \to \infty$ the first Moment is equal to the characteristic time, $M^1 = \tau$. For the finite but large values of $t_{\max}$, we have $M^1 \approx \tau(1 - \frac{(t_{\max}/\tau)^2}{2e^{t_{\max}/\tau}})$, and at $t_{\max}/\tau = 5$ the first Moment equals to $M^1 \approx 0.92\tau$. So, in our case the value of M^1 may serve for approximate estimation of the effective characteristic time constant of the whole expression process.

It is useful to use this approach for crude estimation of characteristic time or durability of expression process after PEF intervention. In such a case we can treat M^1 as a mean characteristic time of liquid expulsion processes reflecting the summarized effect of all the mechanisms in a system. We can define the coefficient of PEF-enhanced durability as

$$\tau^* = M^1_E/M^1_{E=0}. \tag{7}$$

This coefficient shows the degree of the durability increase after the PEF treatment.

C. Influence of field strength E and time t_p of PEF treatment

1. Liquid yield kinetics

Figure 5 presents the curves of excess normalized liquid yield $\Delta Y = Y^{II}_E - Y^{II}_{E=0}$ versus time t at different times of PEF application, t_p, and different values of electric field strength, E. We have applied PEF treatment in different characteristic moments:

- $t_p = 0$ s (before pressing);
- $t_p = 20$ s (initial phase of pressing);
- $t_p = 120$ s (second consolidation phase and specific conductivity of a tissue is low);
- $t_p = 330$ s (press-cake pressure is achieved a constant value);
- $t_p = 600$ s and $t_p = 5400$ s (final consolidation phase).

The range of voltage values used corresponds to conditions of steady PEF-application regime without any disruption of electrical treatment caused by overflow of acceptable maximal current value. For the given pulses protocol, the steady electrical treatment regime was observed for the voltages not exceeding the following maximal values: $U_{max} =$ 600 V for $t_p = 0$ s, $U_{max} = 1000$ V for $t_p = 600$ s, and $U_{max} = 1500$ V for $t_p = 5400$ s.

As can be seen from Fig. 5 the form of the liquid yield Y_E^{II} curves in the time interval of $t < 300$ s practically does not depend on the time of PEF input t_p and the electric field strength values E (that corresponds to small values of deviations $Y_{E\neq 0}^{II} - Y_{E=0}^{II}$). Only at longer time intervals $t > 300$ s in the phase III of consolidation, we can observe a behaviour reflecting the mode of PEF treatment.

At short times of PEF treatment, $t_p \lesssim 100 - 200$ s, the excess liquid yield increases rapidly with E increase as compared with untreated sample (see Fig. 5(a-c)). But ineffectiveness of PEF treatment at short t_p is less in comparison with the cases of later PEF application. It can be explained by the influence of excess quantities of air and extraparticle liquid in the cake pores. The sample is highly water-saturated at earlier period of pre-consolidation and PEF application during this period may cause dielectric breakage and uncontrolled increase of current flow through the system. At such conditions, the intensification degree of PEF treatment Y^* is rather low (see black square for $t_p = 0$ in Fig. 6(a)).

When we apply the PEF treatment later on, for example, at the beginning of the phase III of consolidation, at $t \sim 300 - 400$ s a liquid excess yield seems to be less dependent on E (Fig. 5(d)). Pressure achieves its constant value at this time and most of cells that could be destroyed mechanically at a given pressure (3 bars in our experiments) are already disrupted and most of liquid is evacuated from these cells. The residual isolated intact cells are connected with electrodes through the network of channels containing conductive moisture. At such conditions, the transmembrane potential on intact cells should be high enough, even at low values of E, and, therefore, the effective electropermeabilization of cell can be attained even at minimal values of electric field intensity ($E = 170$ V cm^{-1} in our experiments).

As we can see from comparison of typical micrographs of apple tissue structure received for pressing with and without electrical treatment (Figs. 3(b) and (c)), PEF application at $t = 330$ s ($E = 500$ V cm^{-1}) causes almost complete destruction of the material. The similar pictures are observed in the wide interval of $E \sim 250 - 500$ V cm^{-1} and micrographs allow us to identify only certain quantity of single isolated intact cells.

Dependencies of the coefficient of PEF enhanced durability τ^* versus electric field strength, E (Fig. 6(b)) substantiate conclusions set forth above. We see that the value of τ^* depends considerably on the electric field strength E only at small time of PEF input ($t_p < 100 - 200$ s). The best liquid excess yield as compared with untreated sample may be obtained at the lowest applied field E when the PEF is applied at an instant when the pressure in the system reaches a constant value ($t_p = 300 - 400$ s).

2. Specific conductivity σ, flow rate v and pressure P kinetics

Figure 7 presents the experimental curves of (a) a specific electrical conductivity, σ, and (b) instantaneous flow rate, v, versus time t for the compressed layer of apple slices. The PEF treatment was applied at $t = t_p = 120$ s at different external field strengths, E. As can be seen from Fig. 7(a), the $\sigma(t)$ values begin to rise abruptly after PEF treatment, and this behaviour becomes more pronounced with increasing E. Such rise of $\sigma(t)$ values corresponds to the combined effect of mechanical rupture of cells and their electrical permeabilization.

We present schematically the model of PEF treatment with and without pressing in Fig. 8. In the absence of mechanical pressure the effect of the hidden (or passive) electrical breakdown can be rather important. The electrical conductivity of the whole system depends not only on the destruction degree of individual cells, but also on the character of their connectivity and the presence of continuously conducting channels.

At first, after PEF treatment the real degree of electrical breakdown is hidden and does not affect the conductivity of the whole system. PEF treatment permeabilizates cell membranes and intensifies diffusion processes. The time of electric conductivity build-up in a cellular material after PEF treatment can be estimated from the time constant of diffusion processes: $\tau_D \sim d^2/6D \approx 1$ s, where $d \approx 100$ is a mean cell dimension, and $D \approx 10^{-9}$ m^2 s^{-1} is a diffusion

coefficient of an endocellular fluid. In general, this effect can exhibit a wide distribution of time constants τ_D because of differences in cell dimensions, diffusivity of intracellular solutions, degree of cell permeabilization, etc. Moreover, it can be masked by another related phenomena of resealing process. Heinz et al. (1999) observed that the insulating properties of the cell membranes can be recovered within a few seconds after the small power PEF treatment and it results in decreasing of a cellular system conductivity.

Simultaneous effect of pressing and PEF treatment($P \neq 0$ and $E \neq 0$) can cause the primary changes. First of all, mechanical and electrical stresses can be coupled to cause membrane breakdown in cells (Akinlaja & Frederick, 1998). Then, the external pressure enhances flowing of the fluid from destroyed cells to extracellular and extraparticle channels. All these decrease the retardation time of electric conductivity build-up after PEF treatment. Under simultaneous PEF treatment and material compression we can also eliminate or diminish the effect of a hidden electric breakdown and to depress the cells resealing processes. So, in a general case, we should observe in final state $\sigma_{P\neq 0} > \sigma_{P=0}$. This conclusion is confirmed by the data on $\sigma(t)$ kinetics at $P = 0$ presented in Fig. 7(a) by the dashed line. In this experiment we have dropped the pressure after PEF treatment. This results in considerable decrease of $\sigma(t)$ values as compared with the curve obtained at $P \neq 0$ (Fig. 8).

A liquid flow rate v also depends upon the degree of cellular system destruction as a result of PEF treatment. But the behaviour of $v(t)$ after PEF application does not change drastically (Fig. 7(b)). We can explain such behaviour by the fact of substantial decrease and termination of liquid expulsion from the press-cake by that time. The most pronounced peak is observed only at the highest electric field strength ($E = 400$ V cm^{-1}, Fig. 7(b)), and behaviour of the $v(t)$ curve reveals only increase of its long time tails with increase of E .

Figure 9 presents the experimental curves of (a) reduced pressure values $P^* = P/P_{max}$ and (b) pressure difference $P_0 - P_E$ versus time t for different durations of pre-compression stage before the PEF application. It can be seen that PEF treatment diminishes tissue rigidity which corresponds to the decrease of an effective pressure in a system. Electrical treatment during the pressing period up to 330 s delays pressure increase and accelerates the liquid yield. PEF application ($t_p > 330$ s) can decrease steady-state pressure abruptly and initiate a rise of a liquid flow rate. The peaks of pressure decrease become sharper with pre-compression time increase. It corresponds to the more rapid destruction of cellular material pre-compressed during a longer time. But then, at the final stage, the mechanical properties of a press-cake reflate and pressure increases again up to the maximal level.

D. Optical properties of expressed liquid

The expressed liquid (apple juice) absorption (or coloration) and transmittance change in the course of a simple pressing process, due to filtration properties of the cellular material in the course of time. In the final phase of consolidation (phase III, see Fig. 2), the liquid coloration reduces considerable as compared with coloration of an initial portion of moisture. As we have demonstrated before (Vorobiev, Bazhal & Lebovka, 2000), the simultaneous pressing and electric field treatment result in considerable reduction of coloration of those differential portions of liquid which were obtained after PEF application. Studying the temporal dependencies of optical properties in differential liquid for different modes of pressing and PEF application is *per se* of great interest.

Here we will discuss only the optical properties of the cumulative expressed liquid, which is obtained as a result of combined pressing and PEF treatment at the final stage of the process. Figure 10 presents dependencies of absorption and transmittance versus electric strength E for extracted liquid at different values of t_p. On the one hand, the PEF treatment decreases the liquid coloration, as can be seen from absorption curves in Fig.10. This is a positive factor of PEF treatment. We can explain this phenomena by improvement of the tissue filtration properties during pressing. Moreover, filtration properties of the PEF treated press-cake also get improved with t_p increase because of increase of the pressed tissue consolidation. On the other hand, transmittance of expressed liquid reduces with t_p decrease and increase of E. This is an undesirable phenomena. It can be explained by the influence of electrical treatment on the press-cake filtration properties. It is known that PEF application causes many defects of the tissue. Application of the PEF treatment increases yield of a liquid with high contents of suspended particles.

That's why it is so important to choose a proper instant for PEF application allowing to obtain the cumulative liquid with low coloration and high transmittance. The PEF application at a moment when pressure in the system achieves a constant value is consistent with requirement of the best quality of a juice.

V. CONCLUSION

Investigations of the moisture expression from fine-cut apple raw material under simultaneous mechanical expression and PEF treatment were done. All experiments were performed using both laboratory filter-press cell and high voltage

pulse generator which provided monopolar pulses of rectangular shape. The PEF treatment was applied to materials that were expressed at time $t = t_p$. Then the yield of liquid was analysed in comparison with that of untreated material. The experimental results were obtained for electric field strength E varying from 200 to 1500 V cm^{-1}, pulse duration $t_i = 100$ s, pulse repetition time $\Delta t = 10$ ms, number of pulses $N = 50$, constant pressure $P = 3$ bars and maximal time of mechanical expression $t_{max} = 5.4 \times 10^3 - 10^4$ s.

The summary of results is as follows:

(1) The data obtained allows us to conclude that all kinetics curves ($\sigma(t)$, $Y(t)$, $v(t)$ and $P(t)$) clearly reflect three main consolidation phases in cellular material.

(2) The combination of pressing and PEF treatment gives the most optimum results and permits to enhance significantly the liquid yield in comparison with samples untreated by PEF. The PEF treatment application permits to intensify pressing process whenever the PEF is applied. But best liquid excess yield results at lowest value of applied field E may be obtained when PEF is applied after some pre-compression period. Such pressure pretreatment before PEF application is necessary for structuring uniformity of the press-cake, removing excess moisture and decreasing the electrical conductivity of cellular material. In our study, the pre-compression period duration of $300 - 400$ s was optimal and for which the pressure in the system reaches a constant value. The PEF application in this moment of time results in the best quality of the expressed liquid (apple juice), which is confirmed by its coloration and transmittance.

(3) The simultaneous pressure and PEF treatment application reveals the passive form of the electrical damage. Electrical damage under a low field without pressure application develops very slow. The pressure provokes damage of defected cells, enhances diffusion migration of moisture and depresses cells resealing processes.

(4) The proposed unified approach for liquid yield data analysis allows to reduce the data scattering caused by the differences in the quality of samples.

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FIG. 1. Experimental set-up.

FIG. 2. Typical experimental curves for different reduced properties: liquid yield Y_r, instantaneous flow rate v_r, pressure P_r and specific electrical conductivity σ_r versus time of the expression t. For the convenience of presentation all properties here are reduced to their maximal values, e.g., $Y_r = Y/Y_{max}$, etc., flow rate is determined as $v = dY/dt$. The error bars represent standard deviations of data. At the top we show the proposed scheme of changes in the cellular tissue structure with time.

FIG. 3. SEM micrographs of apple tissue: (a) before treatment, (b) after pressing (P = 3 bars, t = 600 s), (c) after simultaneous PEF & pressing treatment (P = 3 bars, t_p = 330 s, E = 500 V cm^{-1}, t = 600 s).

FIG. 4. Execution steps in the procedure of experimental curves normalization: examples of real experimental curves of expressed liquid yield $Y(\%)$ versus time t obtained for E = 520 V cm^{-1} and t_p = 600 s (a); definition of the first normalized form of liquid yield Y_E^I (b); and definition of the second normalized form of liquid yield Y_E^{II} (c).

FIG. 5. Kinetics of normalized excess liquid yield $\Delta Y = Y_E^{II} - Y_{E=0}^{II}$ at different values of $t_p$and E. The error bars represent standard deviations in the data.

FIG. 6. Degree of PEF treatment intensification Y^* (a) and coefficient of PEF enhanced durability τ^* (b) versus electric field strength E at different values of PEF treatment application time t_p. The error bars represent standard deviations in the data.

FIG. 7. Specific electrical conductivity, σ, (a) and instantaneous flow rate v (b) versus time of expression, t, at t_p = 120 s and different values of E. The error bars represent standard deviations in the data.

FIG. 8. Schematic of the model of PEF treatment with and without pressure P. The conductivity of the sample is higher for case when the pressure is applied.

FIG. 9. Reduced pressure $P^* = P/P_{max}$ (a) and pressure difference $P_{E=0} - P_E$ (a) versus time of expression, t, at different values of t_p and E. The error bars represent standard deviations in the data.

FIG. 10. Absorbance and transmittance of liquid yielded during pressing versus electric field strength, E, at different values of PEF treatment application time, t_p. The error bars represent standard deviations in the data.

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